Figures

Figure 1. Strategies followed in the given examples

Figure 1.1 Ex vivo monitoring of immune response against tetanus toxoid.

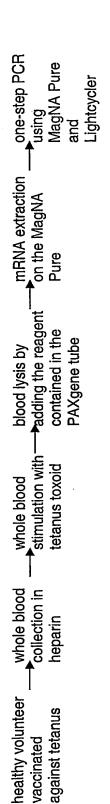
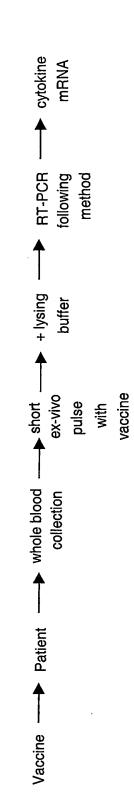


Figure 1.2 Strategy followed in example 3.

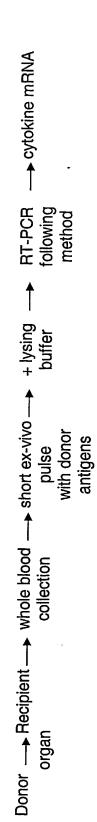


cytokine mRNA

RT-PCR following method + lysing buffer whole blood collection Recipient organ Donor

Figure 1.3 Strategy followed in example 4

Figure 1.4 Strategy followed in example 5.





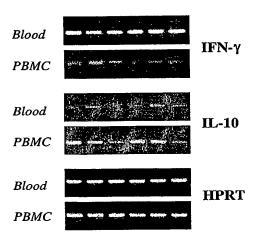


Figure 2.1

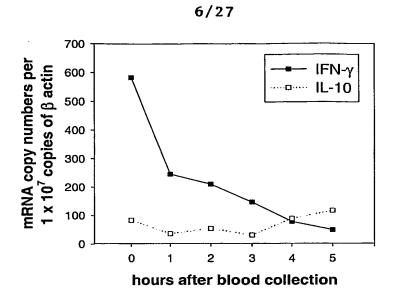


Figure 2.2

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Figure 3:

Blood sampling in PAXgene Tube = 2.5 ml blood + 6.9 ml stabilising reagent, from which the inventors took = 1) 4.7 ml (thus containing 1.25 ml of blood) for Qiagen extraction or

2) 0.4 ml (thus containing 0.11 ml of blood) for MagNA Pure extraction

After centrifugation, the nucleic acid pellet was:

1) PAXgene + Qiagen kit

...washed in water and dissolved in buffer BR1 from the PAXgene blood RNA Kit (Qiagen). Extraction of total RNA was performed as described in the PAXgene blood RNA Kit Handbook

total RNA concentration measured by optical density. 500 ng (and thus different volumes depending on the concentration) used for reverse transcription. This latter and real time PCR were performed as described (Stordeur et al, J Immunol Methods, 259 (1-2): 55-64, 2002)

Results: see Table 4.1

RECOMMENDED PROCEDURE

2) PAXgene + MagNA Pure

... dissolved in the lysis buffer contained in the MagNA Pure mRNA isolation kit. Extraction was performed on the MagNA Pure instrument as recommended by Roche

no need to measure mRNA concentration. 5 μI were used for reverse transcription and real time PCR performed in one step, using the LC RNA Master hybridisation kit (Roche). Real time PCR conditions as described (Stordeur et al, J Immunol Methods, 259 (1-2): 55-64, 2002)

Results: see Table 4.2

PROPOSED PROCEDURE

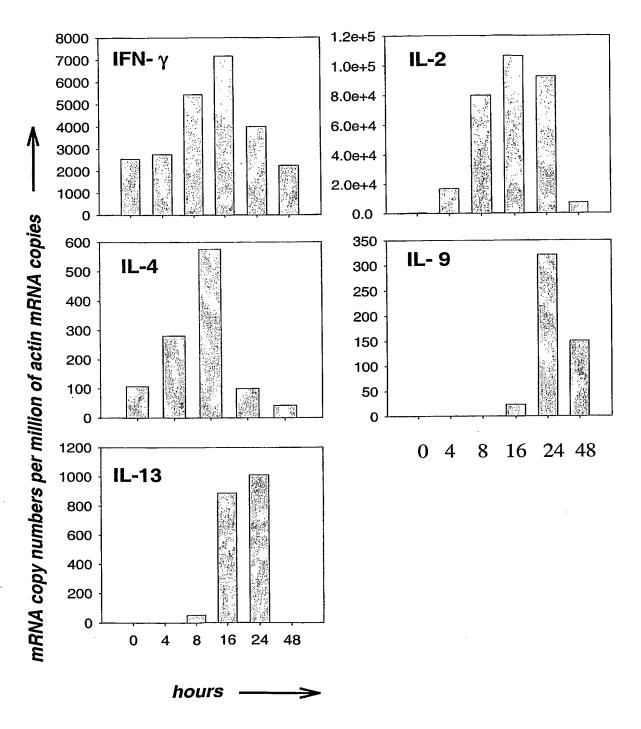
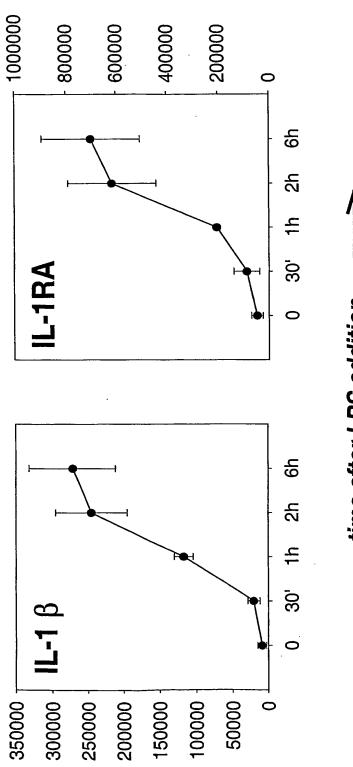


Figure 4



time after LPS addition

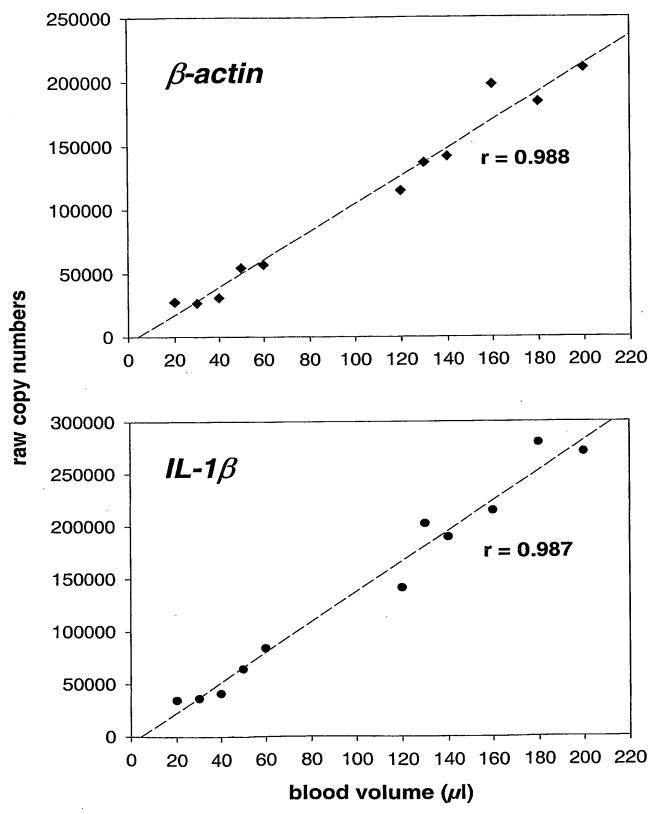
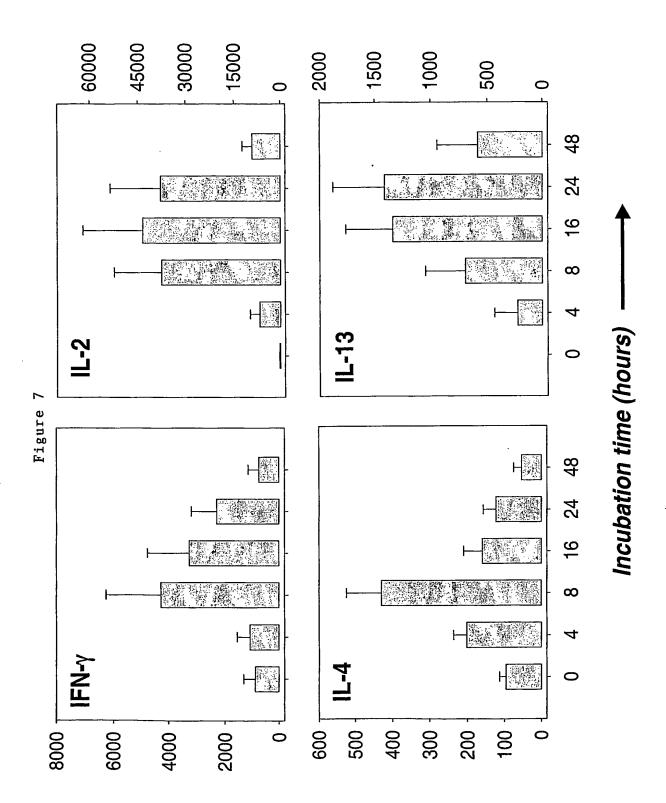
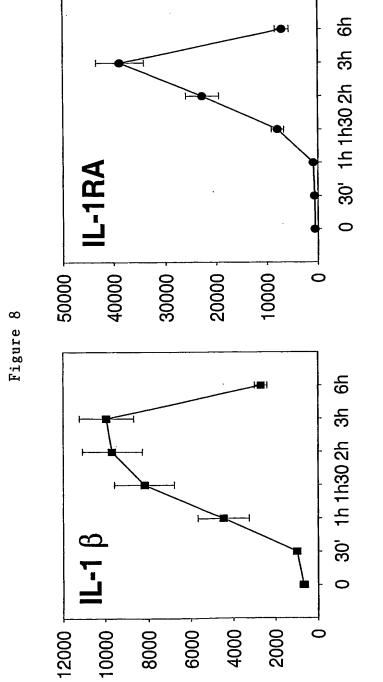


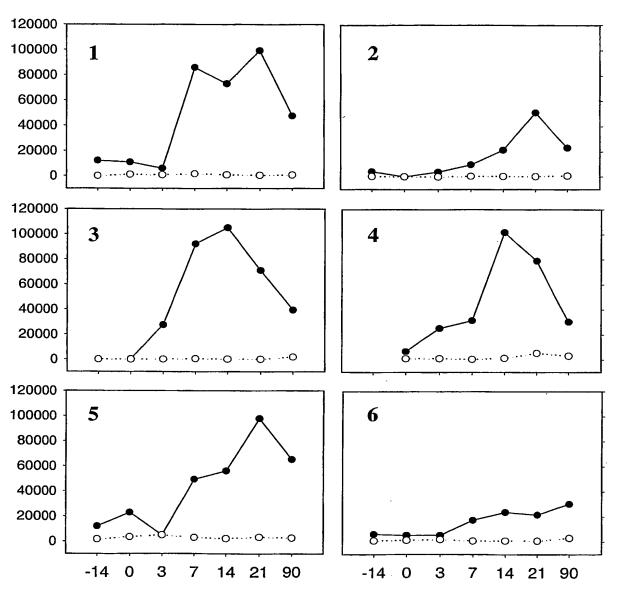
Figure 6





time after LPS injection ———>

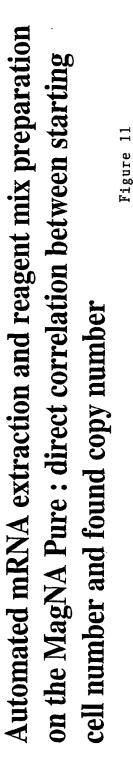
Figure 9

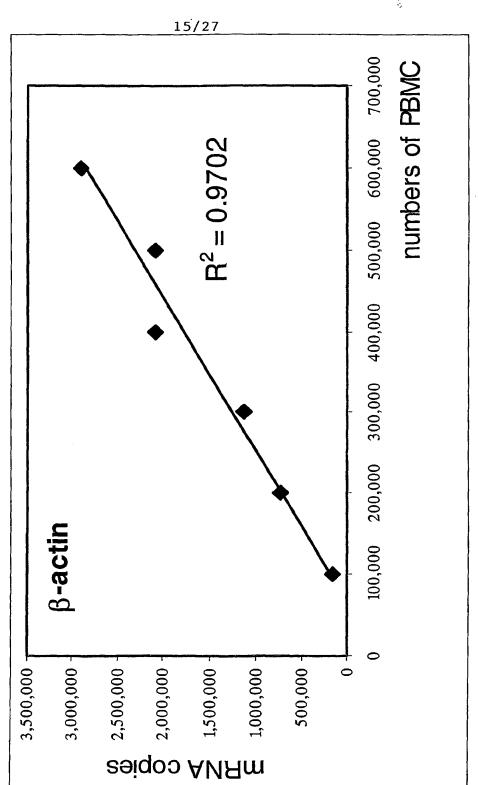


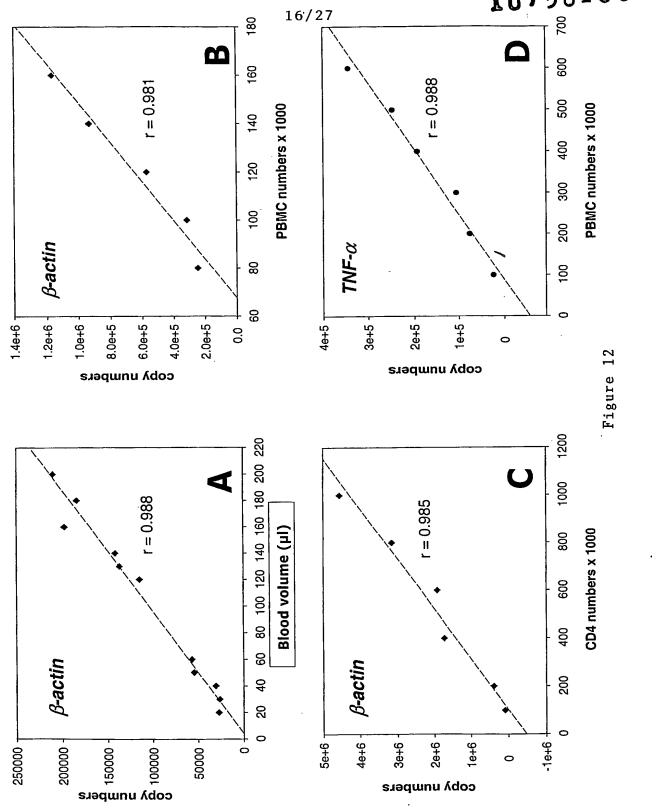
days before / after vaccine administration

Procedure to analyse blood cytokine mRNA expression

- Whole blood sample incubation in the presence the tested potential immuno-modulator (antigen, allergen, cell, xenobiotic,...)
- Blood cells lysis and mRNA stabilisation using the reagent contained in the PAXgene tube
- Automated mRNA extraction and preparation of the reaction mixture for one-step RT-PCR using an automated device (Magna Pure instrument – Roche Diagnostics)
- Quantification of cytokine mRNA levels by real-time PCR using the Lightcycler instrument (Roche Diagnostics)







Immune monitoring in cancer

immunotherapy

Patient #3

7/99: melanoma of the right scapula. Excision

8/01: SC M+ arm, back, abdomen, right testis

4/02: right orchidectomy

4/02: pre-vaccine check-up

5/02: initiation of the vaccine program

Vaccine: MAGE-3 recombinant protein + adjuvant

using the Magna Pure and the

Lightcycler

to assess immune response to MAGE-3 In vitro stimulation of whole blood

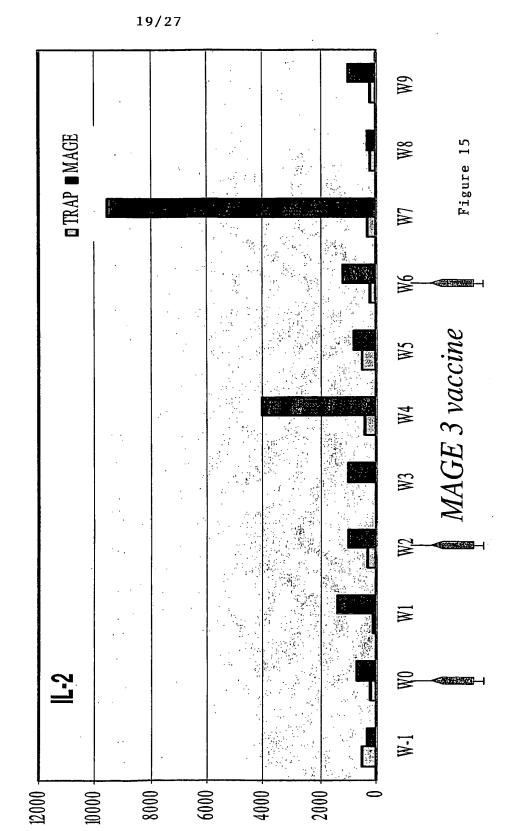
mRNA extraction and RT-PCR - $200 \mu l$ whole blood overnight incubation with MAGE-3 or TRAP antigen once a week during heparinized blood 9 weeks

Patient #3 received 3 injections of anti-MAGE-3 vaccine (MAGE-3 + adjuvant)

IL-2 mRNA in whole blood following MAGE-3 vaccination in Patient #3

IL-2 mRNA

(copy numbers per million of Bactin mRNA copies)



3. E.N.

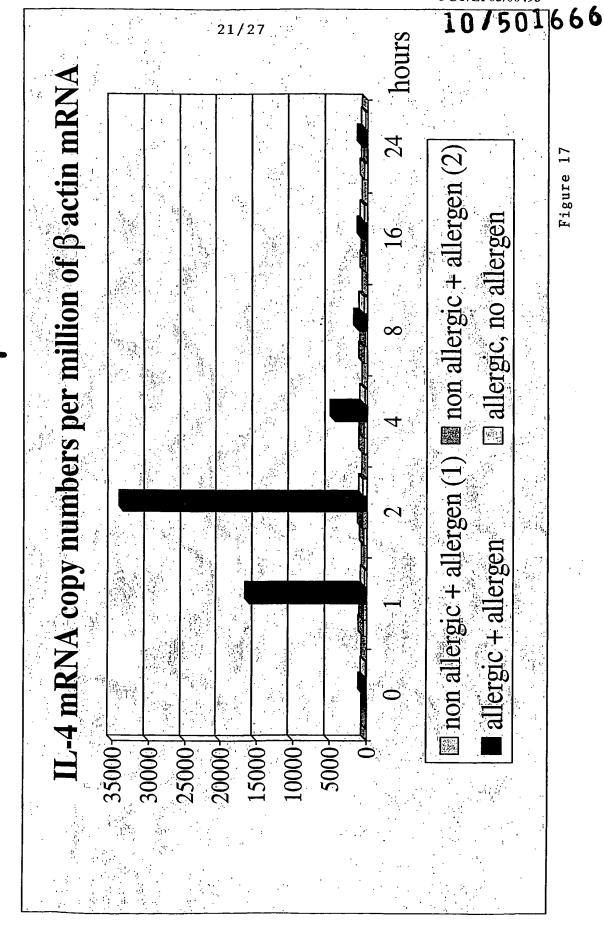
evaluation of immune response to allergen In vitro stimulation of whole blood:

+ PAXgene tube's reagent, + cat allergen (Feld1) after different time periods of culture ▶ heparinized blood patient allergic to the cat or

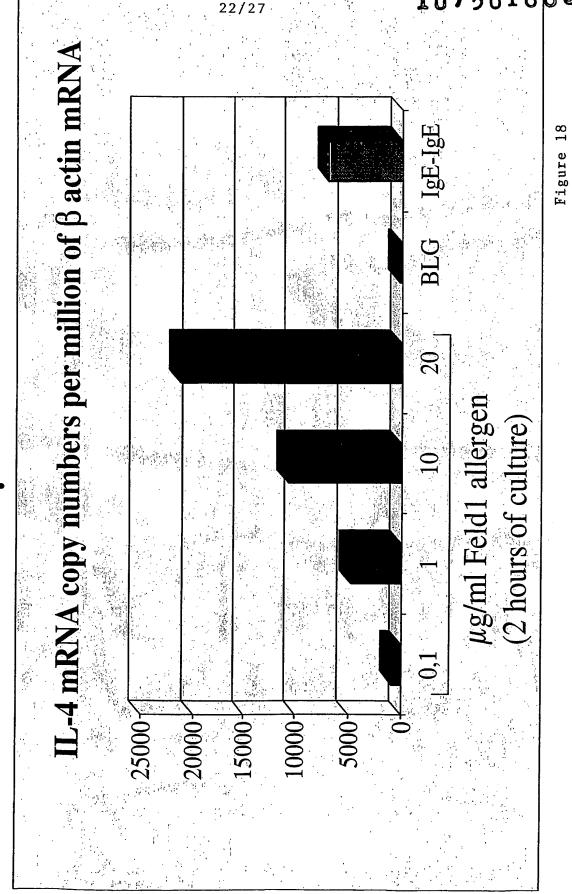
using the MagNA Pure and the mRNA extraction and RT-PCR

non-allergic controls

evaluation of immune response to Feld1 In vitro stimulation of whole blood

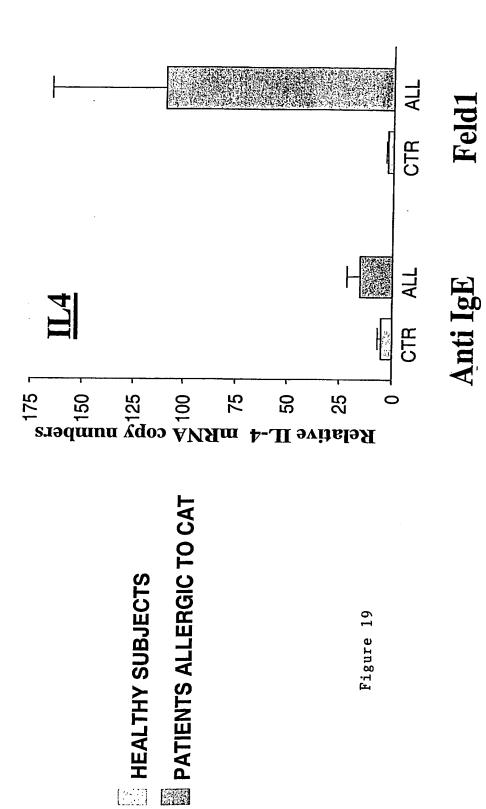


In vitro stimulation of whole blood dose-response to Feld1

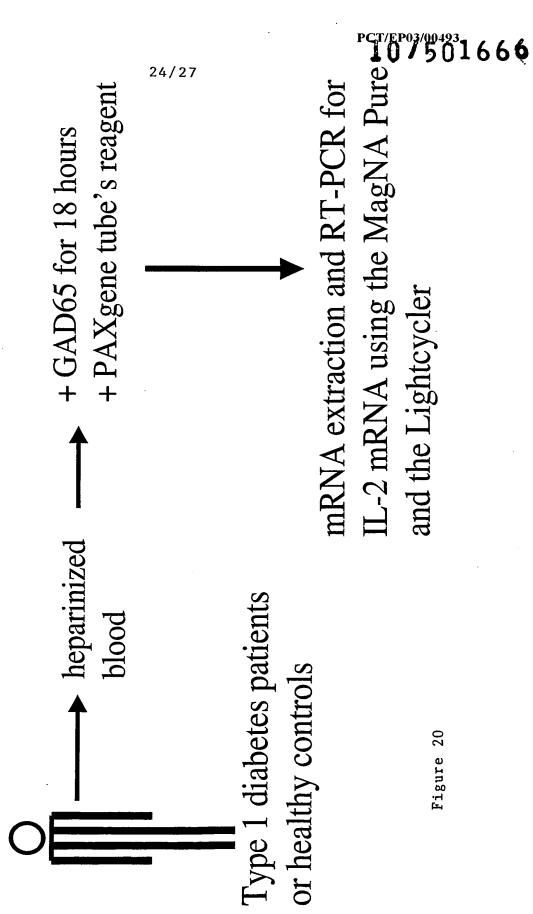


· Lucit in the

stimulation with Feld1 are higher in allergic patients compared to healthy controls IL-4 mRNA levels after whole blood

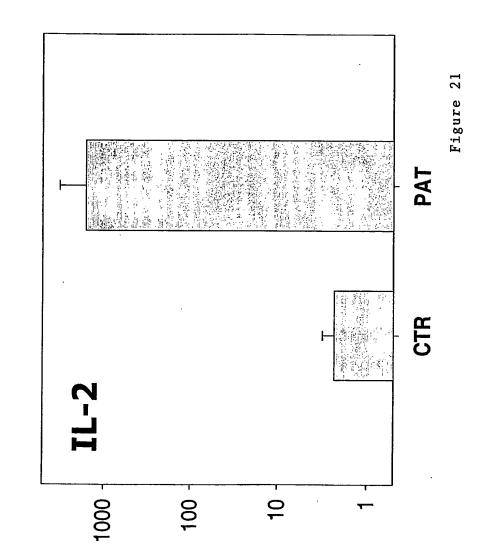


assessment of T cell response to GAD65 In vitro stimulation of whole blood



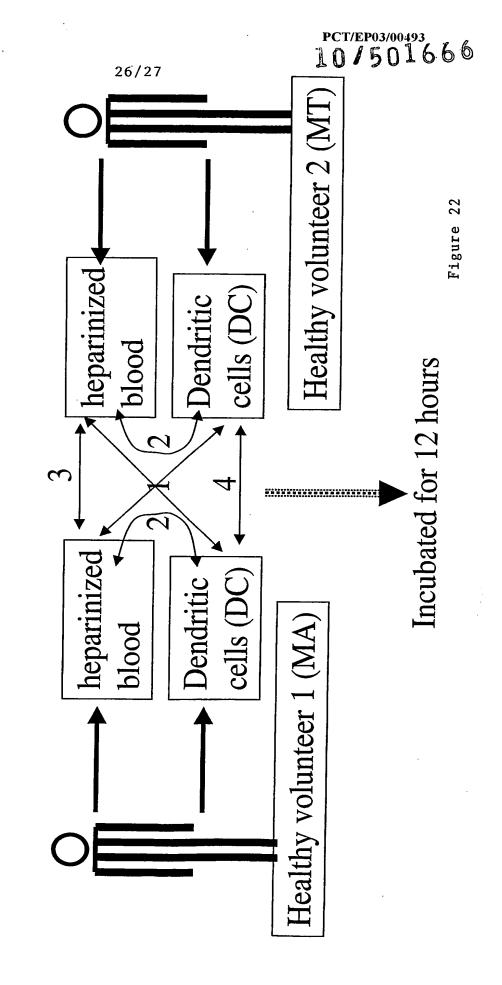
to GAD65 In vitro stimulation of whole blood cell response assessment of

IL-2 mRNA relative copy numbers (Logarithmic scale)

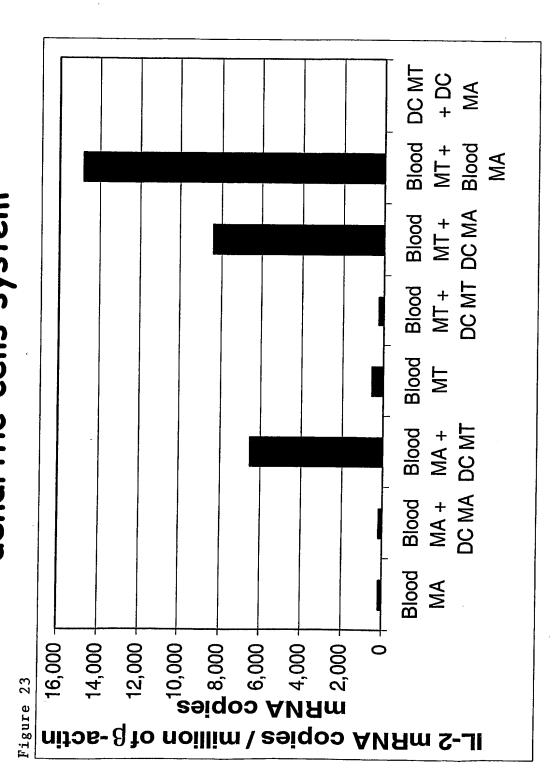


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quantification of IL-2 mRNA in a whole blood Monitoring of alloreactive immune response dendritic cells system



.... V. NU



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